Attorney's Docket No. 35718/237005 (5718-118) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Abad *et al.*	Confirmation No.:	5409
Appl. No.:	10/032,717	Group Art Unit:	1638
Filed:	October 23, 2001	Examiner:	A.R. Kubelik
For:	GENES ENCODING NOVEL BACILLUS THURINGIENSIS PROTEINS WITH PESTICIDAL ACTIVITY AGAINST COLEOPTERANS		

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APPEAL BRIEF UNDER 37 CFR § 1.192

This Appeal Brief is filed pursuant to the "Notice of Appeal to the Board of Patent Appeals and Interferences" filed March 1, 2004.

1. ***Real Party in Interest.***

The real party in interest in this appeal is E.I. du Pont de Nemours and Company, the assignee of the above-referenced patent application.

2. ***Related Appeals and Interferences.***

There are no related appeals and/or interferences involving this application or its subject matter.

3. ***Status of Claims.***

Claims 1-3, 9-12, 17-19, 38-40, 42-46, 48-52, and 54-64 are the subject of this appeal. Claims 4-8, 13-16, 20-27, 41, 47, and 53 have been cancelled.

4. ***Status of Amendments.***

An Amendment After Final was filed on February 2, 2004, to make changes to the claims pursuant to suggestions by the Examiner and to place the claims in better condition for further prosecution. An Advisory Action was mailed February 18, 2004, indicating that the proposed amendments would not be entered.

5. ***Summary of the Invention.***

Pesticidal proteins, nucleic acids encoding them, and methods of use are provided (see, *e.g.*, specification at page 3, 5). The compositions and methods of the invention find use in agriculture for controlling pests of crop plants, particularly Coleopteran insect pests. More specifically, the invention relates to methods of controlling insects utilizing nucleic acids derived from δ-endotoxin genes to produce transformed microorganisms and plants that express a pesticidal polypeptide of the invention.

6. ***Issues.***

Issue 1—Whether claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, 54, and 55-64 meet the enablement requirement of 35 U.S.C. § 112, first paragraph.

Issue 2—Whether claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, 54, and 55-64 meet the written description requirement of 35 U.S.C. § 112, first paragraph.

Issue 3—Whether claims 42, 48, and 54 are anticipated by the prior art and thus are invalid under 35 U.S.C. §102(b).

Issue 4—Whether claims 42, 48, and 54 are indefinite under 35 U.S.C. § 112, second paragraph.

Issue 5—Whether the proposed amendments submitted in the Amendment After Final of February 2, 2004, include new matter.

7. ***Grouping of Claims.***

Applicants believe that the claims do not stand or fall together. Specifically, Applicants believe that claims 1-3, 9-12, 17-19, 38-40, 43-46, 49-52, and 55-64 (the "sequence identity claims") could be considered separately patentable from claims 42, 48, and 54 (the "hybridization claims"). The "sequence identity claims" (claims 1-3, 9-12, 17-19, 38-40, 43-46, 49-52, and 55-64) contain limitations that require the nucleotide sequence of the claims to share a specified percent of sequence identity to SEQ ID NO:1. In contrast, the "hybridization claims" (claims 42, 48, and 54) contain limitations that require the nucleotide sequence of the claims to hybridize to SEQ ID NO:1 under specified hybridization conditions. Because the limitations of the sequence identity claims and the limitations of the hybridization claims place different

constraints on the claimed subject matter, these groups of claims do not necessarily stand or fall together.

Moreover, some of the sequence identity claims differ from each other in the minimum percent sequence identity that is required and may therefore be found to differ in meeting the requirements of patentability. That is, claims 1, 9, and 17 require that the nucleotide sequence has at least 90% sequence identity to SEQ ID NO:1, while claims 38, 43, and 49 contain limitations requiring the nucleotide sequence to have at least 95% identity to SEQ ID NO:1, claims 55, 58, and 63 contain limitations requiring the nucleotide sequence to have at least 93% identity to SEQ ID NO:1, and claims 56, 59, and 64 contain limitations requiring the nucleotide sequence to have at least 94% identity to SEQ ID NO:1. While Applicants believe that all these claims are allowable, it is conceivable that among claims with differing requirements for percent sequence identity, some claims could be found to meet the enablement and written description requirements while others might not. Therefore, the sequence identity claims do not necessarily all stand or fall together. For example, claims requiring at least 90% sequence identity may stand or fall separately from those claims requiring at least 93%, 94%, or 95% sequence identity.

8. ***Argument.***

(a) Issue 1—Whether claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, 54, and 55-64 meet the enablement requirement of 35 U.S.C. § 112, first paragraph.

In the final Office Action (12/03/03, page 2, #3), the Examiner maintained the rejection of claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, and 54 and rejected claims 55-64 under 35 U.S.C. §112, first paragraph, because:

> the specification, while being enabling for nucleic acids encoding SEQ ID NO:2 and 10, expression cassettes comprising the nucleic acid, plants and seeds comprising a construct comprising the nucleic acid and a method of using it to impact a plant pest, does not reasonably provide enablement for any nucleic acid that has 90% identity to SEQ ID NO:1, that hybridizes to SEQ ID NO:1 or that is antisense to a nucleic acid with 90% identity to SEQ ID NO:1....

This rejection will be discussed separately with regard to the sequence identity claims and the hybridization claims.

i) The sequence identity claims meet the enablement requirement

The enablement rejection encompassed claims 1-3, 9-12, 17-19, 38, 43, 46, 49, 52, and 55-64. These claims contain limitations that require the nucleotide sequence of the claims to share a specified percent of sequence identity to SEQ ID NO:1 and thus are referred to herein as the "sequence identity claims." The enablement rejection in the final Office Action only referred to the claim limitation that requires nucleotide sequences to have at least 90% sequence identity to SEQ ID NO:1, but the Advisory Action clarified that the enablement rejection also applied to claims specifying at least 93%, 94%, and 95% sequence identity (*i.e.*, claims 55, 58, and 63 (93%), claims 56, 59, and 64 (94%), and claims 38, 43, and 49 (95%)). Applicants respectfully traverse this rejection and submit that the Examiner is applying an extraordinarily high standard of enablement to the present claims, a standard that is not properly based on case law or on the statute.

Support is provided for the limitations of the sequence identity claims

First, guidance is provided as to what sequence alterations may be made and still provide a pesticidal polypeptide encompassed by the claim. As discussed further below, endotoxin genes are well known in the art. Applicants have provided the exemplary nucleotide sequence of SEQ ID NO:1 and the exemplary amino acid sequence of SEQ ID NO:2. The claimed sequences of the invention vary from these sequences by structural parameters (*i.e.*, percent sequence identity to SEQ ID NO:1; encoding the amino acid sequence set forth in SEQ ID NO:2). Guidance for determining percent identity of sequences is provided in the specification on pages 33 through 38. Moreover, the independent sequence identity claims (*i.e.*, claims 1, 9, and 17) specify that the nucleotide sequence encodes a polypeptide which is pesticidal for at least one pest belonging to the order Coleoptera; therefore, these claims (and the claims dependent on them) encompass functional variants. Guidance regarding alterations that allow the sequence to retain the specified pesticidal activity is also provided (see, *e.g.*, p. 18 (providing guidance regarding conservative substitutions of amino acids) and pp. 19-20 (discussing the activity of variants)). Methods for assaying the pesticidal activity of proteins are routine in the art and are also described in the specification, for example, on pages 8 and 29 and in the experimental section in working examples such as Example 4 (pp. 65-66), Example 6 (p. 67), and Example 7 (p. 69). These working examples teach methods for assaying pesticidal activity of proteins and demonstrate results obtained using these assays. In this manner, Applicants have provided

guidance regarding what changes may be made to allow the endotoxin sequence to retain the specified pesticidal activity.

B. thuringiensis δ-endotoxins are well-known in the art, and further support and guidance is provided by working examples

The Examiner concludes that "[t]he specification provides no guidance as to which amino acids of SEQ ID NO:2 are critical for function." Applicants respectfully disagree with this conclusion. As noted above, Applicants have provided the exemplary nucleotide sequence of SEQ ID NO:1 and the exemplary amino acid sequence of SEQ ID NO:2. The claimed sequences of the invention vary from this sequence by structural parameters (*i.e.*, percent sequence identity to SEQ ID NO:1). As discussed extensively in the specification (*e.g.*, pp. 3, 7, 11-12, 15, 24-25), the disclosed exemplary sequence of SEQ ID NO:2 is a *Bacillus thuringiensis* Cry-8-like δ-endotoxin. The *B. thuringiensis* δ-endotoxins are an extremely well-characterized group of proteins. As discussed in the specification at pp. 24-25:

> Many of the δ-endotoxins are related to various degrees by similarities in their amino acid sequences and tertiary structure, and means for obtaining the crystal structures of *B. thuringiensis* endotoxins are well known. Exemplary high-resolution crystal structure solution of both the Cry3A and Cry3B polypeptides are available in the literature. The inventors of the present invention used the **solved structure of the *Cry3A* gene** (Li *et al.* (1991) *Nature* 353:815-821) to produce a homology model of the Cry8 δ-endotoxin disclosed and claimed herein as SEQ ID NO:2 to gain insight into the relationship between structure and function of the endotoxin, and to design the recombinantly engineered proteins disclosed and claimed herein. A combined consideration of the **published structural analyses** of *B. thuringiensis* endotoxins and the reported function associated with particular structures, motifs, and the like indicates that specific regions of the endotoxin are correlated with particular functions and discrete steps of the mode of action of the protein. For example, **δ-endotoxins isolated from *B. thuringiensis* are generally described as comprising three domains, a seven-helix bundle that is involved in pore formation, a three-sheet domain that has been implicated in receptor binding, and a beta-sandwich motif** (Li *et al.* (1991) *Nature*, 305: 815-821).

As discussed in more detail in the specification (see, *e.g.*, p. 25), the inventors made use of this knowledge in the art to design specific mutations in the Cry8-like proteins to enhance their pesticidal activity. This strategy was successful in creating altered endotoxins with

increased toxicity, as demonstrated by the data presented in working Example 6. Thus, as demonstrated by working examples in the specification, those of skill in the art (*i.e.*, the inventors) were able, in view of the extensive knowledge in the art about *B. thuringiensis* δ-endotoxin structure and function, to modify the exemplary wildtype sequences disclosed herein to provide variant endotoxins with enhanced pesticidal activity. In this manner, the data provided in the specification (*e.g.*, Example 6) demonstrate that one of skill in the art would know what amino acids could be changed to provide a protein with pesticidal activity. Accordingly, Applicants submit that adequate guidance is provided as to which amino acids of SEQ ID NO:2 are critical for function.

The data and working examples provided in the specification also demonstrate the enablement of the claimed invention by showing that sequences of the invention that share a relatively low percent identity to the exemplary sequence of SEQ ID NO:1 encode polypeptides that have pesticidal activity against several Coleopteran pests. In Example 4 (specification pp. 65-66), both the full-length endotoxin encoded by SEQ ID NO:1 and a truncated protein encoded by SEQ ID NO:15 were assayed for pesticidal activity against southern corn rootworm. The nucleotide sequence of SEQ ID NO:15 is a truncation of SEQ ID NO:1 which shares about 55% sequence identity with SEQ ID NO:1. In Example 6 (specification pp. 67-69), several truncated proteins were assayed and shown to have pesticidal activity against Colorado potato beetle (see Table 1, p. 68). These truncated proteins included those encoded by SEQ ID NO:15 and SEQ ID NO:19, which share about 55% and 51% identity, respectively, to the exemplary nucleic acid sequence set forth in SEQ ID NO:1 (alignments performed using BLAST with default parameters). As briefly discussed above, Example 6 also provides assay data for a mutated sequence, NGSR1218-1. This NGSR1218-1 mutant includes the amino acid sequence "NGSR" inserted between amino acids 164 and 165 of the truncated endotoxin of SEQ ID NO:16. The nucleotide sequence encoding this mutant (SEQ ID NO:11) shares about 56% sequence identity with the exemplary nucleotide sequence of SEQ ID NO:1, yet as documented by the data provided in Example 6, both proteins have pesticidal activity. In addition to this data, the specification also provides an exemplary maize-optimized sequence (SEQ ID NO:9) which encodes the same pesticidal polypeptide as SEQ ID NO:15 but shares less than 69% sequence identity with it. Thus, the specification is replete with working examples of sequences that share

a relatively low percentage of identity with SEQ ID NO:1 and which encode polypeptides having pesticidal activity. In fact, the percentage of sequence identity shared by the exemplary SEQ ID NO:1 and these sequences in the working examples is much lower than the "at least 90%" of the broadest sequence identity claims.

The Examiner dismisses the working examples provided by Applicants, concluding that the specification teaches only "a fragment," "a single insertion of 4 amino acids in the 669 amino acid long SEQ ID NO:16," and "nucleic acids encoding SEQ ID NO:2" and stating that the specification "is not enabled for nucleic acids that have 90% identity to SEQ ID NO:1 but that do not encode SEQ ID NO:2." Applicants respectfully disagree with this conclusion. Applicants have provided percent identity variants that include both fragments and amino acid changes to the exemplary wildtype sequences of SEQ ID NO:1 and the encoded SEQ ID NO:2 and thus have taught representative species of the genus of sequences having a particular structural relationship to the exemplary wildtype sequences.

The amount of experimentation required to make and use the subject matter of the sequence identity claims is not undue

The Examiner concludes that "undue trial and error experimentation would be required to make the claimed nucleic acids" (Advisory Action, 2/18/04, continuation sheet). The Federal Circuit has repeatedly stated that enablement is not precluded by the necessity for some experimentation, so long as the experimentation needed to practice the invention is not undue, and that a considerable amount of experimentation is permissible if it is merely routine or if the specification provides a reasonable amount of guidance as to how the experimentation should proceed. *Id. In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed Cir 1988). In the instant case, the quantity of experimentation required to practice independent claim 1 amounts to two steps: (1) generating a nucleic acid comprising a nucleotide sequence that has at least 90% sequence identity to SEQ ID NO:1; and (2) assaying the encoded polypeptide for functional activity. Such assays, while known in the art, have further been presented in the specification. One of skill in the art would appreciate that both of these steps are within the skill of those in the art and that this degree of experimentation is not considered undue.

Similarly, the amount of experimentation needed to practice the other sequence identity claims is not undue. For example, independent claim 9 recites a transformed plant comprising a nucleotide construct that has a nucleotide sequence with at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1 and that encodes a polypeptide that is pesticidal for at least one pest belonging to the order Coleoptera. Thus, in addition to the steps required to practice independent claim 1, independent claim 9 requires the transformation of a plant. Plant transformation is routine in the art; thus, the amount of experimentation required to practice claim 9 is not undue. Similarly, in addition to the steps required to practice independent claim 1, the method of independent claim 17 requires that a nucleotide construct be created in which the nucleotide sequence is operably linked to a promoter; that the construct be introduced into a plant or cell thereof; and that an insect pest feeding on said plant or cell is impacted. The performance and/or evaluation required by each of these additional steps is within the skill of those in the art and would not be considered undue experimentation by those in the art. Likewise, the remaining sequence identity claims, which are all dependent on or incorporate the limitations of independent claim 1, 9, or 17, contain additional requirements which are equally within the skill of those in the art.

Applicants note that it is now customary in the art to make and assay a number of sequences for a desired function in order to achieve the best results. For example, common techniques involve what is often referred to as "shuffling," as described for example in U.S. Patent No. 5,837,458, issued November 17, 1998 with inventors Minshull and Stemmer and entitled, "Methods and Compositions for Metabolic and Cellular Engineering." With such techniques, it is common to mutagenize individual sequences or a set of sequences which are then assayed for a desired activity. Such techniques may even make use of a library of sequences which is recursively mutagenized, screened for function using a functional assay, and re-mutagenized in order to find a sequence exhibiting optimal function. Examples of the use of such techniques include: Minshull and Stemmer (1999) *Current Opinion in Chemical Biology* 3:284-290, entitled "Protein Evolution by Molecular Breeding"; and Christians *et al.* (1999) *Nature Biotechnology* 17: 259-264, entitled "Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling." Such experiments are designed and are intended to encompass the generation and testing of a very large number of variant sequences for a desired

function. As indicated by these and other publications in the art, this level of experimentation is now considered routine in the art and thus would not be considered "undue experimentation" under *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed Cir 1988) and *In re Jackson*, 217 USPQ 804, 807 (Bd. Pat. App. & Int. 1982) (holding that a considerable amount of experimentation is permitted to practice the invention and is not undue if it is merely routine in the art or if the specification provides a reasonable amount of guidance and direction to perform such experimentation).

One of skill in the art could make and use the claimed invention without undue experimentation

It is true that some embodiments of the nucleotide sequence which meet the percent identity limitation of the claims may not encode a polypeptide that has the specified pesticidal activity. However, one of skill would readily be able to use the assays taught in the specification to determine which nucleotide sequences that met the sequence identity limitations of the claims also encoded polypeptides having the specified pesticidal activity. Applicants note that the presence of inoperative embodiments within the scope of the claims does not render the claims invalid. *Atlas Powder Co. v. E.I. duPont de Nemours & Co.*, 750 F.2d 1569, 224 USPQ 409 (Fed. Cir. 1984). Nor would the amount of experimentation required to test a particular polypeptide for pesticidal activity be considered undue by one of skill in the art, as evidenced by the assay results presented in the specification, for example, in working Examples 4 (pp. 65-66), 6 (p. 67), and 7 (p. 69). The references cited by the Examiner—Lazar *et al.* (1988) *Mol. Cell. Biol.* 8: 1247-52 and Hill *et al.* (1998) *Biochem. Biophys. Res. Comm.* 244: 573-577—illustrate that one of skill would readily be able to determine whether a particular sequence change affected the function of a protein. Accordingly, one of skill in the art would be able to determine the functionality of polypeptides encompassed by the claimed invention without undue experimentation.

The Federal Circuit has repeatedly stated that enablement is not precluded by the necessity for some experimentation, so long as the experimentation needed to practice the invention is not undue. *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed Cir 1988). Furthermore, a considerable amount of experimentation is permissible, if it is merely routine, or

if the specification provides a reasonable amount of guidance in which the experimentation should proceed. *Id.* Applicants stress that when evaluating the quantity of experimentation required, the court looks to the amount of experimentation required to practice a single embodiment of the invention rather than the amount required to practice every embodiment of the invention as the Examiner implies. For example, in *Wands*, the claims at issue were drawn to immunoassay methods using any monoclonal antibody having a binding affinity for HbsAg of at least 10^{-9} M. The PTO had taken the position that the claim was not enabled because it would take undue experimentation to make the monoclonal antibodies required for the assay. The Federal Circuit reversed and held that the claims were enabled, as the amount of experimentation required to isolate monoclonal antibodies and screen for those having the correct affinity was not undue. *See Id.* Clearly, the Federal Circuit did not contemplate that every antibody useful in the methods of the claim must be identified. Rather, the court considered the amount of experimentation required to identify one or a few monoclonal antibodies having the required affinity. *See also, Johns Hopkins University v. Cellpro*, 931 F. Supp. 303, 324 (D. Del. 1996), *aff'd in part, vacated in part, and remanded*, 47 USPQ2d 1705 (Fed. Cir. 1998) (stating that "[t]he specification need only enable one mode of making the claimed invention.").

Thus, for the reasons discussed above, Applicants respectfully submit that the sequence identity claims meet the enablement requirement of 35 U.S.C. §112, first paragraph. Based on the knowledge in the art and the guidance provided in the specification, the skilled artisan could choose among possible sequence modifications to produce polypeptides within the sequence identity parameters set forth in the claims and then test these sequence variants to determine if they retained pesticidal activity. The amount of experimentation needed to perform such an evaluation would not be considered by those of skill in the art to be undue; therefore, the amount of guidance presented in the specification is sufficient to enable the claims. Accordingly, Applicants respectfully submit that the Examiner's rejection of the sequence identity claims 1-3, 9-12, 17-19, 38-40, 43-46, 49-52, and 55-64 under 35 U.S.C. §112, first paragraph, for lack of enablement should be reversed.

ii) The hybridization claims meet the enablement requirement

Applicants are unsure of the exact grounds for the enablement rejection of the hybridization claims (*i.e.*, claims 42, 48, and 54). These claims were rejected for lack of enablement in the Office Action of 3/12/03; in that Action, the Examiner stated (p. 5) that "the specification fails to provide guidance for the exact hybridization or amplification conditions and probes/primers to use in isolation of nucleic acids other than SEQ ID NOs: 1 and 3." In Applicants' Amendment of 5/29/03, claims 42, 48, and 54 were amended to add hybridization and wash conditions. Despite these amendments, the rejection of these claims was maintained in the Final Office Action (dated 12/03/03), but the basis for this rejection was not discussed. Therefore, Applicants are uncertain of the exact basis for this rejection, but will discuss the rejection as it might have been applied.

Applicants note that claims 42, 48, and 54 specify hybridization conditions and wash conditions. Support for these limitations is found in the specification on page 32, lines 1-4. Hybridization techniques are very well established in the art. As indicated on page 32, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. Thus, if one of skill desired the stringency of the wash conditions to be varied, the temperature or salt concentration would be altered. As evidence of this common understanding in the art, Applicants provided an excerpt from Moore and Dowhan, "Preparation and Analysis of DNA: Hybridization Analysis of DNA Blots," *Current Protocols in Molecular Biology*, 2002, Chapter 2.10.11, Supplement 26, John Wiley and Sons, New York. The excerpt concludes that the stringency of wash conditions are manipulated based on the T_m calculation and that the manipulation of stringency occurs via a change in temperature or salt concentration. This approach allows for the high level of reproducibility seen in the art as it relates to hybridization assays.

> *Designing washes for heterologous hybridization.* Calculations of T_m become more critical if heterologous probing is being attempted. If the aim is to identify sequences that are merely related, not identical, to the probe (*e.g.*, members of a multigene family, or a similar gene in a second organism), then it is useful to have an idea of the degree of mismatching that will be tolerated by a "moderate-" or "low-" stringency wash. The best way to approach this is to first establish the lowest temperature at which only homologous hybridization occurs with a particular SSC

> concentration. Then assume that 1% mismatching results in a 1°C decrease in the T_m (Bonner *et al.*, 1973) and reduce the temperature of the final wash accordingly (for example, if sequences with ≥90% similarity with the probe are being sought, decrease the final wash temperature by 10°C). If the desired degree of mismatching results in a wash temperature of <45°C, then it is best to increase the SSC concentration so that a higher temperature can be used. Doubling the SSC concentration results in a ~17°C increase in T_m, so washes at 45°C in 0.1xSSC and 62°C in 0.2xSSC are roughly equivalent. Note that in these extreme cases it may also be necessary to reduce the hybridization temperature to as low as 45°C (aqueous solution) or 32°C (formamide solution).

This excerpt is found in Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York), as incorporated by reference into the specification on pp. 32-33. In view of the knowledge of the art, one of skill would recognize that a wash would be maintained until equilibrium was reached. One of skill would recognize that the length of the wash recited in claims 42, 48, and 54 is carried out for at least the length of time it is required to establish equilibrium. Wash time extending beyond equilibrium will not influence the outcome. Thus, the recitation of a specific temperature and salt concentration clearly define the stringency of the wash conditions.

Further, as also cited in the specification on pp. 32-33, extensive guides to the hybridization of nucleic acids are found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), all of which are incorporated by reference into the specification (see p. 77). Accordingly, given the state of the art, Applicants respectfully submit that one of skill would be able to identify a nucleic acid comprising a nucleotide sequence that met the hybridization limitation of claims 42, 48, and 54.

The Examiner states that:

> Lack of recitation of wash time means that very short wash times are encomapassed [*sic*] by the claims, and in very short wash times DNAs of varying similarity to the reference DNA hybridize. Furthermore, as any lab researcher who has ever forgotten about a washing blot knows it is

> very possible to wash off the probe; at equilibrium no detectable probe is left.

Applicants respectfully disagree with these statements as a basis for the rejection of the hybridization claims for failure to meet the written description requirement. First, those of skill in the art routinely include controls to ensure that an experiment has worked properly. This routine safeguard would protect against the concern stated by the Examiner that very short wash times could give misleading results. Moreover, as discussed in the specification at page 31, under stringent conditions, a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (*e.g.*, at least 2-fold over background). Applicants respectfully disagree with the Examiner's conclusion that "at equilibrium no detectable probe is left"; probe will be removed only if the wash conditions and degree of sequence identity favor dissociation. Under specified wash conditions and with a probe of known composition, reproducible results are obtained.

Claims 42, 48, and 54 also contain limitations requiring that the nucleic acids comprise a nucleotide sequence that encodes a polypeptide having pesticidal activity against at least one pest belonging to the order Coleoptera. As discussed extensively above, assays for pesticidal activity are known in the art and are also taught in detail in the specification. Thus, one of skill would be able to determine whether a particular nucleic acid met the hybridization requirement of the claims as well as the pesticidal activity requirement of the claims. Other requirements of these claims are routine in the art and would also not require undue experimentation. Accordingly, Applicants respectfully submit that claims 42, 48, and 54 meet the enablement requirement of 35 U.S.C. §112 and request that the Examiner's rejection of these claims be reversed.

(b) Issue 2—Whether claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, 54, and 55-64 meet the written description requirement of 35 U.S.C. § 112, first paragraph.

In the final Office Action (12/03/03, page 4, #4), the Examiner maintained the rejection of claims 1-3, 9-12, 17-19, 38, 42, 43, 46, 48, 49, 52, and 54 and rejected claims 55-64 under 35 U.S.C. §112, first paragraph:

> [A]s containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that

> the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is repeated for the reasons of record.... Applicant's arguments...have been fully considered but they are not persuasive."

This rejection will be discussed separately with regard to the sequence identity claims and the hybridization claims.

i) The sequence identity claims meet the written description requirement

The written description rejection in the final Office Action encompassed claims 1-3, 9-12, 17-19, 38, 43, 46, 49, 52, and 55-64. These claims contain limitations that require the nucleotide sequence of the claims to share a specified percent of sequence identity to SEQ ID NO:1 and thus are referred to herein as the "sequence identity claims." In maintaining this rejection, the Examiner disregards not only Applicants' arguments but also the case law cited in those arguments. Applicants respectfully submit that the Examiner is applying an extraordinarily high standard of written description to the present claims, a standard that is not properly based on case law or on the statute.

As an initial matter, Applicants note that the written description rejection in the final Office Action only referred to the claim limitation that requires nucleotide sequences to have at least 90% sequence identity to SEQ ID NO:1, but some of the claims included in the rejection specify at least 93%, 94%, and 95% sequence identity (*i.e.*, claims 55, 58, and 63 (93%), claims 56, 59, and 64 (94%), and claims 38, 43, and 49 (95%)). Presumably these claim limitations have also been considered by the Examiner and the basis for rejection would be the same as stated for the 90% sequence identity claims, *mutatis mutandis*.

Also, Applicants note that in the final Office Action (12/03/03, page 4, #4, 3d paragraph), the Examiner stated that "nucleic acids that have 90% identity to SEQ ID NO:1 ***are predictable***, nucleic acids that have 90% identity to SEQ ID NO:1 AND that encode pesticidal proteins are not" (emphasis added). Thus, the written description rejection is on the grounds that there is inadequate description of sequences that both meet the sequence identity requirement of the claims and also meet the functional requirement (*i.e.*, that the encoded polypeptide has pesticidal activity).

The sequence identity claims meet the written description requirement as articulated by the Federal Circuit

Applicants respectfully submit that the present claims and specification meet the written description requirement of 35 U.S.C. §112, first paragraph, as clarified by *University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1569, 43 USPQ2d 1398 (Fed. Cir. 1997) and *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991); *cert. denied* 112 S.Ct. 169 (1991). Applicants have provided exemplary sequences of the invention as set forth in SEQ ID NO:1. The claimed nucleic acids are defined in relation to the nucleotide sequence of SEQ ID NO:1; that is, the claimed nucleic acids comprise nucleotide sequences that share a specified percentage of sequence identity with SEQ ID NO:1. Applicants have thus provided a structural definition of the sequences of the invention. Applicants have also provided assays by which those of skill in the art can readily assess whether a nucleic acid molecule meeting the nucleotide sequence element of the claims also meets the functional limitation element of the claims. This is what *Eli Lilly* requires, and Applicants have also conceived the sequences of the invention as articulated in *Amgen*; that is, Applicants are able "to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it." *Amgen*, 18 USPQ2d at 1021.

Applicants further note that the Federal Circuit has explicitly stated that:

> *Eli Lilly* did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.

Amgen, Inc. v. Hoechst Marion Roussel, Inc., 314 F.3d 1313, 1332, 65 USPQ2d 1385, 1398 (Fed. Cir. 2003). *See also, Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1320, 66 USPQ2d 1429, 1438 (noting that "[i]n more recent cases, however, this court has distinguished *Lilly*" and further noting that in *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 323 F.3d 956 (Fed. Cir. 2002), "neither the specification nor the deposited biological material recited the precise 'structure, formula, chemical name, or physical properties' required by *Lilly*.")

B. thuringiensis δ-endotoxins are well-known in the art, and further support is provided in the specification with working examples

As discussed extensively in the specification (*e.g.*, on pp. 3, 7, 11-12, 15, 24-25), the disclosed exemplary sequence of SEQ ID NO:2 is a *Bacillus thuringiensis* Cry-8-like δ-endotoxin. The *B. thuringiensis* δ-endotoxins are an extremely well-characterized group of proteins. As discussed in the specification at pp. 24-25:

> The inventors of the present invention used the **solved structure of the *Cry3A* gene** (Li *et al.* (1991) *Nature* 353:815-821) to produce a homology model of the Cry8 δ-endotoxin disclosed and claimed herein as SEQ ID NO:2 to gain insight into the relationship between structure and function of the endotoxin, and to design the recombinantly engineered proteins disclosed and claimed herein. A combined consideration of the **published structural analyses** of *B. thuringiensis* endotoxins and the reported function associated with particular structures, motifs, and the like indicates that specific regions of the endotoxin are correlated with particular functions and discrete steps of the mode of action of the protein. For example, **δ-endotoxins isolated from *B. thuringiensis* are generally described as comprising three domains, a seven-helix bundle that is involved in pore formation, a three-sheet domain that has been implicated in receptor binding, and a beta-sandwich motif** (Li *et al.* (1991) *Nature*, 305: 815-821).

As discussed in more detail in the specification on p. 25, the inventors made use of this knowledge to design specific mutations in the Cry8-like proteins to enhance their pesticidal activity. This strategy was successful in creating altered endotoxins with increased toxicity, as demonstrated by the data presented in working Example 6. Thus, the inventors were able, in view of the extensive knowledge in the art about *B. thuringiensis* δ-endotoxins, to modify the exemplary wildtype sequences disclosed herein to provide an endotoxin with enhanced pesticidal activity. In this manner, the data provided in the specification (*e.g.*, Example 6) demonstrate that one of skill would know what amino acids could be changed to provide a protein with pesticidal activity. Accordingly, Applicants submit that adequate guidance is provided as to which amino acids of SEQ ID NO:2 are critical for function and that therefore, Applicants have envisioned the detailed construction of the gene to distinguish it from other materials, thereby meeting the written description requirement.

Applicants note that in the final Office Action, the Examiner stated that "Applicant has not described even one nucleic acid that ... has 90% identity to SEQ ID NO:1 AND that encodes a pesticidal protein." Applicants respectfully disagree with this conclusion. The specification teaches a number of nucleic acids that share relatively low percent sequence identity with SEQ ID NO:1 but encode proteins having pesticidal activity. In Example 4 (specification pp. 65-66), both the full-length endotoxin encoded by SEQ ID NO:1 and a truncated protein encoded by SEQ ID NO:15 were assayed for pesticidal activity against southern corn rootworm. The nucleotide sequence of SEQ ID NO:15 is a truncation of SEQ ID NO:1 which shares about 55% sequence identity with SEQ ID NO:1. In Example 6 (specification pp. 67-69), several truncated proteins were assayed and shown to have pesticidal activity against Colorado potato beetle (see Table 1, p. 68). These truncated proteins included those encoded by SEQ ID NO:15 and SEQ ID NO:19, which share about 55% and 51% identity, respectively, to the exemplary nucleic acid sequence set forth in SEQ ID NO:1 (alignments performed using BLAST with default parameters). Another mutant assayed for pesticidal activity in Example 6 was NGSR1218-1 (encoded by SEQ ID NO:11). The NGSR1218-1 mutant includes the amino acid sequence "NGSR" inserted between amino acids 164 and 165 of the truncated endotoxin of SEQ ID NO:16. The nucleotide sequence encoding this mutant (SEQ ID NO:11) shares about 56% sequence identity with the exemplary nucleotide sequence of SEQ ID NO:1, yet both encoded proteins have pesticidal activity. The specification also teaches an exemplary maize-optimized sequence (SEQ ID NO:9), which encodes the same pesticidal polypeptide as SEQ ID NO:15 but shares less than 69% sequence identity with it. Thus, the present specification provides multiple working examples illustrating the production of sequences that encode pesticidal proteins and share a relatively low percentage of sequence identity with SEQ ID NO:1. Multiple working examples are presented, illustrating that Applicants were in possession of the claimed invention at the time of filing.

In light of the above statements, Applicants respectfully assert that the present claims and specification satisfy the statutory written description requirement. Accordingly, Applicants respectfully request that the rejection of the sequence identity claims under 35 U.S.C. §112, first paragraph, be reversed.

ii) The hybridization claims meet the written description requirement

In the final Office Action (of 12/03/03, page 4), the Examiner rejected claims 42, 48, and 54 for failing to meet the written description requirement, stating that "Applicant has not described nucleic acids that hybridize to SEQ ID NO:1 AND that encode pesticidal proteins." (emphasis in original) The Office Action continued, "Applicant has not described even one nucleic acid that hybridizes to SEQ ID NO:1 AND that encodes a pesticidal protein ...." Applicants respectfully disagree with the conclusion that the hybridization claims do not meet the written description requirement.

Applicants note that claims 42, 48, and 54 recite hybridization and wash conditions. Support for these limitations is found in the specification (see, *e.g.*, p. 32, lines 1-4). Hybridization techniques are very well established in the art. As indicated on page 32, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. Thus, if one of skill desired the stringency of the wash conditions to be varied, the temperature or salt concentration would be altered. As evidence of this common understanding in the art, Applicants provide an excerpt from Moore and Dowhan, "Preparation and Analysis of DNA: Hybridization Analysis of DNA Blots," *Current Protocols in Molecular Biology*, 2002, Chapter 2.10.11, Supplement 26, John Wiley and Sons, New York. The excerpt from Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York), which is incorporated into the specification by reference, concludes that the stringency of wash conditions are manipulated based on the T_m calculation and that the manipulation of stringency occurs via a change in temperature or salt concentration. This approach allows for the high level of reproducibility seen in the art as it relates to hybridization assays.

> *Designing washes for heterologous hybridization.* Calculations of T_m become more critical if heterologous probing is being attempted. If the aim is to identify sequences that are merely related, not identical, to the probe (*e.g.*, members of a multigene family, or a similar gene in a second organism), then it is useful to have an idea of the degree of mismatching that will be tolerated by a "moderate-" or "low-" stringency wash. The best way to approach this is to first establish the lowest temperature at which only homologous hybridization occurs with a particular SSC

> concentration. Then assume that 1% mismatching results in a 1°C decrease in the T_m (Bonner *et al.*, 1973) and reduce the temperature of the final wash accordingly (for example, if sequences with ≥90% similarity with the probe are being sought, decrease the final wash temperature by 10°C). If the desired degree of mismatching results in a wash temperature of <45°C, then it is best to increase the SSC concentration so that a higher temperature can be used. Doubling the SSC concentration results in a ~17°C increase in T_m, so washes at 45°C in 0.1xSSC and 62°C in 0.2xSSC are roughly equivalent. Note that in these extreme cases it may also be necessary to reduce the hybridization temperature to as low as 45°C (aqueous solution) or 32°C (formamide solution).

Further, as cited in the specification on pp. 32-33, extensive guides to the hybridization of nucleic acids are found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), all of which are incorporated by reference into the specification (see p. 77). Accordingly, given the state of the art, Applicants respectfully submit that one of skill would be able to identify a nucleic acid comprising a nucleotide sequence that met the hybridization condition limitations of claims 42, 48, and 54.

The Examiner concludes that:

> Lack of recitation of wash time means that very short wash times are encomapassed [*sic*] by the claims, and in very short wash times DNAs of varying similarity to the reference DNA hybridize. Furthermore, as any lab researcher who has ever forgotten about a washing blot knows it is very possible to wash off the probe; at equilibrium no detectable probe is left.

Applicants respectfully disagree with these conclusions. First, those of skill in the art routinely include controls in experiments to ensure that the experiment has worked properly. This routine safeguard would protect against the concern stated by the Examiner that very short wash times could give misleading results. In addition, as discussed in the specification at page 31, under stringent conditions, a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (*e.g.*, at least 2-fold over background). Further, Applicants respectfully disagree with the Examiner's conclusion that "at equilibrium no

detectable probe is left"; probe will be removed only if the wash conditions and degree of sequence identity favor dissociation. Under specified wash conditions and with a probe of known composition, reproducible results are obtained.

In view of the knowledge of the art, one of skill would recognize that a wash would be maintained until equilibrium was reached and thus one of skill would recognize that the length of the wash recited in claims 42, 48, and 54 is carried out for at least the length of time it is required to establish equilibrium. Wash time extending beyond equilibrium will not influence the outcome. Thus, the recitation of a specific temperature and salt concentration clearly define the stringency of the wash conditions.

Claims 42, 48, and 54 also contain limitations requiring that the nucleic acids have pesticidal activity. Because, as discussed above, assays for pesticidal activity are taught in the specification and are also known in the art, Applicants respectfully submit that these claims meet the written description requirement of 35 U.S.C. §112 and are in condition for allowance.

In view of the above discussion, Applicants respectfully submit that all of the claims meet the written description of 35 U.S.C. §112 and are in condition for allowance. Accordingly, the rejection by the Examiner of the hybridization claims for failing to meet the written description requirement should be reversed.

(c) Issue 3—Whether claims 42, 48, and 54 are anticipated by the prior art and thus are invalid under 35 U.S.C. §102(b).

The final Office Action (12/03/03, page 7, #6) rejected claims 42, 48, and 54 under 35 U.S.C. §102(b) as anticipated by Michaels *et al.* (1996, U.S. Pat. No. 5,554,534). This rejection was discussed in the Advisory Action, in which the Examiner stated that Applicants' arguments [that the claims were not anticipated by Michaels *et al.*] were "not found persuasive because wash times are not recited."

This rejection is respectfully traversed. Claims 42, 48, and 54 recite high-stringency hybridization and wash conditions, as supported in the specification (see, *e.g.*, p. 32). Applicants note that the sequence search results cited by the Examiner indicated a "query match" between SEQ ID NO:1 and the Michaels sequence of only 70.8%, and sequences sharing only 70.8% sequence identity would not be expected to hybridize to each other under the high stringency

conditions set forth in the claims. Specifically, employing the equation of Meinkoth and Wahl as described in the specification on page 32 in view of the specified hybridization and wash conditions and the sequence of SEQ ID NO:1, the melting temperature of a probe consisting of SEQ ID NO:1 is 66.45°C[1]. Under the wash conditions specified in claims 42, 48, and 54 (*i.e.*, a wash in 0.1x SSC at 60°C to 65°C), the hybrid of a nucleic acid consisting of SEQ ID NO:1 and the Michaels *et al.* sequence would not be stable. Accordingly, claims 42, 48, and 54 are not anticipated by Michaels *et al.* and the rejection of these claims over the Michaels reference under 35 U.S.C. §102(b) should be reversed.

(d) Issue 4—Whether claims 42, 48, and 54 are indefinite under 35 U.S.C. § 112, second paragraph.

The final Office Action (12/03/03, page 6, #5) rejected claims 42, 48, and 54 as being indefinite. Applicants responded with an amendment to specify a time of hybridization and a wash temperature (as supported in the specification, for example, on p. 32), but the Examiner did not enter the amendment. Accordingly, this discussion is based on the claims as they stood before the proposed amendment.

Claims 42, 48, and 54 recite hybridization and wash conditions. Support for these limitations is found in the specification (see, *e.g.*, p. 32, lines 1-4). Hybridization techniques are very well established in the art. As indicated on page 32, specificity is typically the function of

[1] The Meinkoth and Wahl formula for melting temperature (p. 32, lines 8-11) is: $T_m = 81.5°C + 16.6$ (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. As specified in claims 42, 48, and 54, the hybridization conditions are such that the molarity of monovalent cations is 1; %GC of SEQ ID NO:1 is 38%, the concentration of formamide is 50%; the length of SEQ ID NO:1 is 3621 nucleotides. Accordingly, the melting temperature of a hybrid of the probe of SEQ ID NO:1 with its complement under the specified hybridization conditions is 66.45°C. As noted on page 32, lines 13-15, Tm is reduced by about 1°C for each 1% of mismatching, and it is known in the art that the Tm is reduced by about 1.4°C for each 1% of mismatching. Thus, a sequence sharing 70.8% sequence identity with SEQ ID NO:1 would be expected to have a melting temperature with SEQ ID NO:1 of about 37.25°C. However, the wash conditions specified in claims 42, 48, and 54 require that the wash is performed in 0.1xSSC at 60°C to 65°C. 0.1x SSC has a monovalent cation concentration of 0.0165M, which according to the Meinkoth and Wahl formula would provide an effective melting temperature of 70.4°C. Because, as is known in the art, the Tm is reduced by about 1°C to 1.4°C for each 1% of mismatch, washes performed under these conditions would result in removal of probes sharing less than about 96% sequence identity (% homology = 100-[70.4-65)/1.4] = 96.1%). The hybrid of SEQ ID NO:1 with the sequence reported by Michaels *et al.* would not be expected to be stable under these wash conditions.

post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. Thus, if one of skill desired the stringency of the wash conditions to be varied, the temperature or salt concentration would be altered. As evidence of this common understanding in the art, Applicants provide an excerpt from Moore and Dowhan, "Preparation and Analysis of DNA: Hybridization Analysis of DNA Blots," *Current Protocols in Molecular Biology*, 2002, Chapter 2.10.11, Supplement 26, John Wiley and Sons, New York. This excerpt from Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York) is incorporated by reference into the specification on p. 32. The excerpt concludes that the stringency of wash conditions are manipulated based on the T_m calculation and that the manipulation of stringency occurs via a change in temperature or salt concentration. This approach allows for the high level of reproducibility seen in the art as it relates to hybridization assays.

> *Designing washes for heterologous hybridization.* Calculations of T_m become more critical if heterologous probing is being attempted. If the aim is to identify sequences that are merely related, not identical, to the probe (*e.g.*, members of a multigene family, or a similar gene in a second organism), then it is useful to have an idea of the degree of mismatching that will be tolerated by a "moderate-" or "low-" stringency wash. The best way to approach this is to first establish the lowest temperature at which only homologous hybridization occurs with a particular SSC concentration. Then assume that 1% mismatching results in a 1°C decrease in the T_m (Bonner *et al.*, 1973) and reduce the temperature of the final wash accordingly (for example, if sequences with ≥90% similarity with the probe are being sought, decrease the final wash temperature by 10°C). If the desired degree of mismatching results in a wash temperature of <45°C, then it is best to increase the SSC concentration so that a higher temperature can be used. Doubling the SSC concentration results in a ~17°C increase in T_m, so washes at 45°C in 0.1xSSC and 62°C in 0.2xSSC are roughly equivalent. Note that in these extreme cases it may also be necessary to reduce the hybridization temperature to as low as 45°C (aqueous solution) or 32°C (formamide solution).

Further, as cited in the specification on pp. 32-33, extensive guides to the hybridization of nucleic acids are found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular*

Biology—Hybridization with Nucleic Acid Probes, Part I, Chapter 2 (Elsevier, New York); and Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), all of which are incorporated by reference into the specification (see p. 77). Accordingly, given the state of the art, Applicants respectfully submit that one of skill would be able to identify a nucleic acid comprising a nucleotide sequence that met the hybridization limitation of claims 42, 48, and 54.

The Examiner concludes that:

> Lack of recitation of wash time means that very short wash times are encomapassed [*sic*] by the claims, and in very short wash times DNAs of varying similarity to the reference DNA hybridize. Furthermore, as any lab researcher who has ever forgotten about a washing blot knows it is very possible to wash off the probe; at equilibrium no detectable probe is left.

Applicants respectfully disagree with these conclusions. First, those of skill in the art routinely include controls in experiments to ensure that the experiment has worked properly. This routine safeguard would protect against the concern stated by the Examiner that very short wash times could give misleading results. In addition, as discussed in the specification at page 31, under stringent conditions, a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (*e.g.*, at least 2-fold over background). Applicants respectfully disagree with the Examiner's conclusion that "at equilibrium no detectable probe is left"; probe will be removed only if the wash conditions and degree of sequence identity favor dissociation. Under specified wash conditions and with a probe of known composition, reproducible results are obtained.

In view of the knowledge of the art, one of skill would recognize that a wash would be maintained until equilibrium was reached. One of skill would recognize that the length of the wash recited in claims 42, 48, and 54 is carried out for at least the length of time it is required to establish equilibrium. Wash time extending beyond equilibrium will not influence the outcome. Thus, the recitation of a specific temperature and salt concentration clearly define the stringency of the wash conditions. Accordingly, the rejection of claims 42, 48, and 54 for being indefinite under 35 U.S.C. §112, second paragraph, should be reversed.

Although the above arguments have been made as if the proposed amendments submitted in the Amendment After Final were not entered, Applicants respectfully submit that the proposed amendments also clearly define the hybridization and wash conditions required by the claims. As discussed below under heading (e), support is provided in the specification for the proposed amendments. Moreover, one of skill is able to assess the thermal melting point of a particular nucleic acid hybrid using knowledge in the art as well as guidance provided in the specification, including the references incorporated into the specification on pages 32-33. Accordingly, whether the proposed amendments to the claims are entered or not, Applicants respectfully submit that the claims meet the requirements of 35 U.S.C. §112, second paragraph.

(e) Issue 5—Whether the proposed amendments submitted in the Amendment After Final of February 2, 2004, include new matter.

In the Advisory Action of February 18, 2004, the Examiner refused to enter amendments that Applicants had proposed in their Amendment After Final of February 2, 2004, asserting that the proposed amendments constituted new matter. Applicants respectfully disagree with this conclusion.

The proposed amendments to claims 42, 48, and 54 specify that hybridization occurs for 12 hours and that the wash in 0.1x SSC is performed at 4°C lower than the thermal melting point of the hybrid of said first nucleotide sequence and said second nucleotide sequence (i.e., SEQ ID NO:1), wherein said thermal melting point is between 60°C to 65°C. Support for these proposed amendments is provided in the specification. Specifically, page 32, lines 3-4, state that "[t]he duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours"; thus, the specification provides *in haec verba* support for the hybridization time limitation. With regard to the proposed wash temperature limitation, page 32, line 3 specifies that high stringency conditions include a wash in 0.1x SSC at 60°C to 65°C, and page 32, lines 19-20 specify that stringent conditions "can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point...." Thus, the specification provides support for the limitation that the wash is performed at 4°C lower than the thermal melting point of the hybrid, wherein said thermal melting point is between 60°C and 65°C.

The Examiner concludes in the Advisory Action that "[t]here is no support in the specification for washing at 4C below 60-65C (i.e., 56-61C). Pg 32-33 of the specification do not state a thermal melting point temperature of 60-65C for the hybrid." Applicants respectfully disagree with this conclusion, and note that under MPEP §1302.01:

> [E]xact terms need not be used *in haec verba* to satisfy the written description requirement.... *Eiselstein v. Frank*, 52 F.3d 1035, 1038 (Fed. Cir. 1995); *In re Wertheim*, 541 F.2d 257,265 (CCPA 1976). See also 37 CFR 1.121(a)(5) which merely requires substantial correspondence between the language of the claims and the language of the specification.

Applicants respectfully submit that the support in the specification for the proposed claim amendments meets the requirements of the MPEP, the statute, and case law, and therefore the proposed claim amendments do not constitute new matter. Accordingly, the proposed amendments should be entered in the case.

CONCLUSION

In view of the arguments presented above, Applicants contend that each of claims 1-3, 9-12, 17-19, 38-40, 42-46, 48-52, and 54-64 is patentable. Therefore, reversal of the rejections under 35 U.S.C. §102(b) and 35 U.S.C. §112, first and second paragraphs, is respectfully solicited.

Respectfully submitted,

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Lynda-Jo Pixley — June 1, 2004
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